REMARKS

Claims 1-18, 32, 46, 50 and 54 are pending in the application. Claims 12, 18, 32, 50, and 54 remain withdrawn from consideration by the Examiner, in view of the Restriction Requirement made final in this Office Action. Claims 1, 11, 13-17, and 46 are rejected.

Claim 5 has been amended but does not contain new matter. Support for the amendment is found in the specification at least at page 14.

The applicant thanks the Examiner for withdrawing the species election.

Rejection Under the First Paragraph of 35 U.S.C. § 112.

The Examiner has rejected claims 1-11, 13-17, and 46 under 35 U.S.C. § 112, first paragraph, asserting that the claims contain subject matter that was not described in the specification in such a way as to enable one skilled in the art to which it pertains to make and/or use the invention. Specifically, the Examiner has alleged lack of enablement on five grounds:

(1) the claims are not enabled because the KDR1 and KDR2 antibodies recited in claim 11 are allegedly not publicly available;

(2) the specification allegedly does not make clear "how homogenous" the population of natal $CD34^+$ KDR^+primitive stem cells that give rise to both hematopietic and stromal cell populations is;

(3) it is allegedly "not clear" from the specification how the injected human donor post natal CD34+KDR+ cells differentiated into any specific cell type as there is allegedly a lack of characterization of these cells by phenotype or functional capacity;

(4) the specification allegedly does not teach how to extrapolate data obtained from *in vivo* studies wherein post natal $CD34^+KDR^+$ cells were injected in non-immuno-compromised murine blastocytes or injected into the regenerating murine muscle to the development of effective *in vivo* or *in vitro* methods of generating a differentiating human cell that specifics selected type; and

(5) the specification does not reasonably provide enablement for a method of generating a differentiating human cell type of a selected type wherein the stem cell is separated from the differentiating mammalian cell by any porous barrier.

The Examiner makes the general argument that:

> In view of the quantity of experimentation necessary, the unpredictability of the art, the lack of sufficient guidance in the specification, the limited working examples, and the limited amount of direction provided given the breadth of the claims, it would take undue trials and errors [sic] to practice the claimed invention.

The applicant traverses each of these rejections.

With respect to point 1, the applicant first notes that the rejection only applies to claim 11, as claim 11 is the sole claim that recites use of the KDR1 and/or KDR2 antibodies. These particular antibodies are not, as the Examiner states, "required" to practice the invention in its broader aspects, *e.g.*, claim 1. Any means to separate a KDR^+ cell from others cell types known or to be developed the art is acceptable. *See, e.g.,* the Specification at ¶ ¶ 15 and 16.

Moreover, KDR1 and KDR2 antibodies were readily available to a person of ordinary skill in the art at the time the application was filed. The antibodies could be commercially obtained by purchase from, *e.g.,* Sigma-Aldrich Corporation which provided them to the public as products numbers V9134 and V3003. Exemplary product brochures are attached hereto.

Additionally, the production of such antibodies was described in the scientific literature, available to a person of ordinary skill in the art, prior to the earliest priority date of the filing of this invention. The descriptions can be found in, *e.g.*, Simon, M., et al., Receptors of vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) in fetal and adult human kidney: localization and [125] VEGF binding sites. *J. Am. Soc. Nephrol.* **9**, 1032 (1998) and Ziegler, B.L., A key marker defining hematopoietic stem cells *Science* **285**, 1553 (1999), copies of each of which are submitted with the IDS that accompanies this response.

With respect to point 2, above, the Examiner asserts that the specification does not provide disclosure as to the degree of homogeneity of the cell population, and speculates that it is "very possible" that the cell population is heterogeneous, containing cell populations that give rise to both hematopietic and stromal elements. The applicant disagrees with the Examiner's analysis, as the point made is not relevant to the enablement analysis under 35 U.S.C. § 112. The claims require only a cell that is KDR^+. This cell can be obtained from hematopoietic tissues using known techniques. Moreover, the specification very clearly lays out how the cells of the initial hematopoietic cell population are selected and characterized so that those used in the method are KDR^+ cells. *See*, the specification at, *e.g.* paragraphs 66-67, citing a method that was known in the prior art published by Ziegler, *et al.* (1999, Blood 93:3355-3368). Indeed, a

$CD34^+KDR^+$ cells can generate both hematopoietic and endothelial cells, as shown, *e.g.*, in example 1, depending on the nature of the differentiator to which it is subjected, for some $CD34^+KDR^+$ cells are bipotent. *See, e.g.,* Example 1 of the Specification.

With respect to point 3, the Examiner appears to be questioning whether the differentiation recited in the claim actually occurs, contending that there is no characterization of the resultant cells as to phenotype or functional capacity. The applicant disagrees. Example 2 of the specification demonstrates that post-natal $CD34^+KDR^+$ cells are capable of differentiating into endothelial cells as demonstrated by phenotypic analysis (cell markers). In Example 5, it is demonstrated that the cells may be differentiated into human skeletal cell muscles. In view of these examples, a person of skill in the art would have been able to practice the claims at the time the application was filed.

The Examiner contends that a person of skill in the art would not have understood the applicability of the method *in vitro* to an *in vivo* context (point 3, above). The applicant disagrees.

A person of skill in the art, based on the description in the specification and his knowledge would have understood how to make and use the invention both *in vitro* and *in vivo.*

The Examiner contends that the specification does not enable claim 4 where the cell is separated by any porous membrane as recited in claim 4. In view of the amendments made herein, it is submitted that the Examiner's rejection is no longer applicable.

Accordingly, in view of the foregoing, it is respectfully submitted that the Examiner's rejection based upon 35 U.S.C. § 112, first paragraph, have been overcome and/or are no longer applicable. Reconsideration and withdrawal of the rejections are respectfully requested.

Rejection Under First Paragraph 35 U.S.C. § 112 - Written Description.

The Examiner has rejected claims 1-11, 13-17, and claim 46 under 35 U.S.C. § 112, first paragraph, asserting that the claims contain subject matter that is not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors at the time the application was filed had possession of the invention. Specifically, the Examiner contends that the specification fails to describe any reagent other than an antibody that specifically binds with KDR. Therefore, claims reciting the reagent lack support of the written description.

The applicant respectfully traverses the rejection.

First, as a threshold matter, the applicant notes that claims 1-4, 6-10, 13-14, and 46 do not recite use of a reagent that specifically binds with KDR in order to isolate a stem cell from human hematopoietic tissue. The only claims that recite a "reagent" element are claims 5 and 10. The rejection is therefore not applicable to claims 1-4, 6-10, 13, 14, and 46, and its withdrawal is requested.

Moreover, with respect to claims 5 and 10, the applicant disagrees with the Examiner's analysis. The specification considered in combination with the knowledge of a person of ordinary skill in the art at the time the application was filed provides sufficient written description of "reagents that specifically bind to KDR", such that the application is fully compliant with 35 U.S.C. § 112, first paragraph. In particular, the specification teaches that methods of isolating KDR cells can be accomplished using antibodies that bind to KDR or, for example, selecting a cell by making use of an antigen that is co-expressed with KDR, such as VEGF receptor 1 or a VGF receptor 3. As is known to a person of skill in the art, use of this method, for example, would require different antibodies, and/or receptor identification systems using reagents other than a KDR^+ antibody. Accordingly, for at least this reason, claims 5 and 10 are fully compliant with the written description requirement. Reconsideration and withdrawal of the rejection is respectfully requested.

Rejection Under 35 U.S.C. § 103(a).

The Examiner has rejected claims 1-3, 5-10, 13-17, and 46 under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 5,736,396 of Bruder, *et al.*, taken in view of U.S. Patent No. 5,912,133 of Lemischka, and in view of the specification disclosure at page 63, lines 48 and page 4, lines 4-10. In particular, the Examiner asserts that Bruder teaches a method of generating a differentiated cell of a selected type by incubation of human mesechymal stem cells in the presence of differentiating mammalian cells or a "conditioned medium" that are effective to induce the differentiation into a lineage of choice. The Examiner concedes that Bruder does not teach that the stem cells are human KDR^+ stem cells. Lemischka, according to the Examiner, teaches a method of isolating human FLK^+ stem cells using an antibody that specifically binds FLK-1. The Examiner characterizes the specification as teaching that human KDR^+ cells are the "same subpopulation" of $CD34^+$ cells as "human FLK^+ stem cells." Thus, the Examiner reasons

that it would have been obvious to a person of ordinary skill in the art the time the invention was made to apply the teaching of Bruder to that of Lemischka and to substitute isolated human mesechymal stem cells with isolated human KDR^+ stem cells to obtain the claimed method. The motivation to make this combination, according to the Examiner, is derived from the fact that isolated human KDR^+ stem cells can be induced to differentiate *in vitro* or *in vivo.*

The applicant respectfully traverses the rejection.

Lemischka teaches a method for isolating cells that express FLK-1 receptors on their surface, including binding the cells to a polyclonal or monoclonal antibody specific for the FLK-1 receptor, and isolating the cells that are bound to the antibody from cells that are not bound to the antibody. The goal of Lemischka is to provide isolated mammalian nucleic acid molecules that encode protein tyrosine kinases that are expressed in primitive hematopoietic cells and which are not expressed in mature hematopoietic cells. Lemischka does not teach or suggest that the FLK-1 receptor bearing cells can be used in a method of generating human cells of specific types by selected differentiation through use of a fully mature, differentiated mammalian cell of a specific, selected tissue or cell type, nor does it teach or suggest that murine cells having a VEGF receptor on their surfaces would necessarily differentiate along the same pathway as murine cells exhibiting a VEGF receptor (FLK-1).

Bruder teaches methods for *in vitro* or *ex vivo* lineage-directed induction of isolated, culture expanded human mesenchymal stem cells. The method of Bruder includes contacting the mesenchymal stem cells with a bioactive factor effective to induce differentiation into a lineage of choice. Bruder, as the Examiner has conceded, does not teach or suggest use of human KDR^+ stem cells for induction. Bruder, as the Examiner conceded, does not teach or suggest human KDR^+ stem cells for induction. Additionally it does not teach or suggest that cells express a VEGF receptor would be useful in the method of inducing *ex vivo* lineage directed differentiation described in Bruder. Rather, Bruder does not appear to select any subpopulation of HSC's, but rather uses in its process, a large, presumably heterogeneous, population of mesenchymal stem cells obtained from bone marrow. *See*, Example 1.

The combination of Lemischka and Bruder proposed by the Examiner does not render the claimed invention obvious on several grounds. First, the combination does not teach or suggest all elements of the invention. As conceded by the Examiner, Bruder does not teach use of KDR bearing cells, rather he relies upon Lemischka's disclosure of FLK bearing cells to supplement

the deficiency. However, FLK-1 receptors are murine receptors for vascular endothelial growth factors, in contrast to the KDR receptors, which are human receptors. Although the receptors are for similar growth factors, they are not structurally identical, nor are they expressed on the same cells (human cells versus murine cells). Accordingly, the combination proposed by the Examiner does not teach or suggest each element of the invention.

Moreover, particularly in view of this deficiency, a person of ordinary skill in the art would not have been motivated to make the combination proposed by the Examiner, nor would he have had any reasonable expectation that the combination would be successful. Lemischka, as discussed above, does not teach or suggest use of KDR^+ cells in any of the processes described therein. Moreover, Bruder does not teach or suggest that any subpopulation of cells needs to be selected out from the initial, presumably heterogeneous, cell population.

Accordingly, for at least the reasons given above, the combination proposed by the Examiner does not render the claimed invention obvious. Reconsideration and withdrawal of the rejection is respectfully requested.

Respectfully submitted,

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Attachments:

Petition for Extension of Time
Sigma Aldrich product brochures
Article - *J. Am. Soc. Nephrol.* **9,** 1032 (1998)
Article - *Science* **285**, 1553 (1999)